

WE CLAIM:

5 1. A covalent HCV NS4A-NS3 complex comprising the central hydrophobic domain of native HCV NS4A peptide, a linker, and the HCV NS3 serine protease domain, wherein the hydrophobic domain of native HCV NS4A peptide is tethered by the linker to the amino terminus of the HCV NS3 protease domain.

10 2. The covalent HCV NS4A-NS3 complex of claim 1, wherein the linker comprises at least about 4 amino acid residues.

15 3. The covalent HCV NS4A-NS3 complex of claim 2, wherein the linker consists essentially of 4-6 amino acid residues.

4. The covalent HCV NS4A-NS3 complex of claim 3, wherein the linker consists essentially of about 4 amino acid residues.

20 5. The covalent HCV NS4A-NS3 complex of claim 4, wherein the linker has a sequence defined by SEQ ID NO: 21 or SEQ ID NO: 22.

25 6. The covalent HCV NS4A-NS3 complex of claim 5, having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20.

30 7. The covalent HCV NS4A-NS3 complex of claim 1 which is modified by replacement of one or more hydrophobic amino acid residues at position 17 or 18 of the HCV NS3 serine protease domain with a hydrophilic amino acid residue.

8. The covalent HCV NS4A-NS3 complex of claim 7 in which one or more isoleucine residues at position 17 or 18 of the HCV NS3 serine protease domain is replaced by a lysine residue.

35 9. The covalent HCV NS4A-NS3 complex of claim 8, having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-4, 6-8, 10, 12-14 and 16-18.

10. The covalent HCV NS4A-NS3 complex of claim 1 which is modified by replacement of a serine residue at position 139 of the HCV NS3 serine protease domain with an alanine residue.

5

11. The covalent HCV NS4A-NS3 complex of claim 10, having an amino acid sequence selected from the group consisting of SEQ ID NOs: 5-8, 15-18 and 20.

10 12. A nucleic acid encoding a covalent HCV NS4A-NS3 complex, which covalent HCV NS4A-NS3 complex comprises the central hydrophobic domain of native HCV NS4A peptide, a linker, and the HCV NS3 serine protease domain, wherein the hydrophobic domain of native HCV NS4A peptide is tethered by the amino acid linker to the amino terminus of the HCV NS3 protease domain.

15 13. The nucleic acid of claim 12, wherein the linker comprises a least about 4 amino acid residues.

20 14. The nucleic acid of claim 13, wherein the linker consists essentially of 4-6 amino acid residues.

25 15. The nucleic acid of claim 14, wherein the linker consists essentially of 4 amino acid residues.

30 16. The nucleic acid of claim 15, wherein the amino acid linker has a sequence defined by SEQ ID NO: 21 or SEQ ID NO: 22.

35 17. The nucleic acid of claim 16, which encodes a covalent HCV NS4A-NS3 complex having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-20.

18. The nucleic acid of claim 12, which encodes a covalent HCV NS4A-NS3 complex which is modified by replacement of one or more hydrophobic amino acid residues at position 17 or 18 of the HCV NS3 serine protease domain with a hydrophilic amino acid residue.

195

19. The nucleic acid of claim 18 which encodes a covalent HCV NS4A-NS3 complex in which one or more isoleucine residues at position 17 or 18 of the HCV NS3 serine protease domain are replaced by a lysine residue.

5

20. The nucleic acid of claim 19, which encodes a covalent HCV NS4A-NS3 complex having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-4, 6-8, 10, 12-14 and 16-18.

10

21. The nucleic acid of claim 12, which encodes a covalent HCV NS4A-NS3 complex which is modified by replacement of a serine residue at position 139 of the HCV NS3 serine protease domain with an alanine residue.

15

22. The nucleic acid of claim 21, which encodes a covalent HCV NS4A-NS3 complex having an amino acid sequence selected from the group consisting of SEQ ID NOs 5-8, 15-18 and 20.

20

23. A recombinant vector comprising the nucleic acid of claim 12, which vector is capable of directing expression of the nucleic acid.

24. A host cell comprising the recombinant vector of claim 23.

25

25. A method for making a covalent HCV NS4A-NS3 complex comprising culturing the host cell of claim 24 under conditions in which the nucleic acid or vector is expressed.

30

26. A method for identifying an HCV NS3 protease inhibitor, comprising (a) contacting a covalent HCV NS4A-NS3 complex of claim 1 with a peptide substrate and a suspected protease inhibitor under conditions in which proteolysis can occur; and (b) detecting whether the covalent HCV NS4-NS3 complex has cleaved the substrate.

35

27. A method for identifying an inhibitor of the nucleic acid unwinding activity of an HCV NS3 helicase, comprising (a) contacting a covalent HCV NS4A-NS3 complex of SEQ ID NO: 4, 12-19 or 20 with a double stranded RNA substrate and a suspected inhibitor under conditions in which unwinding of the substrate can occur; and (b)

detecting whether and the extent to which the covalent HCV NS4-NS3 complex has unwound the substrate.

28. A method for identifying an inhibitor of an HCV NS3
5 helicase, comprising (a) contacting a covalent HCV NS4A-NS3 complex
of SEQ ID NO: 4, 12-19 or 20 with ATP and a suspected inhibitor under
conditions in which ATP hydrolysis can occur; and (b) detecting whether
the covalent HCV NS4-NS3 complex has exhibited ATPase activity.

10

15

20150727 14:00:00